Recovery of Oxidative Phosphorylation in Rat Liver Mitochondria after Whole Body Irradiation*

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There have been many conflicting reports on the effects of x-rays in vitro and in vivo on respiration and phosphorylation in various animal tissues. Kusnets (1) reported a decrease in spleen respiration within 30 minutes after rats had received whole body irradiation of 800 r, and Sullivan and Dubois (2) recorded a similar decrease with 400 r which reached a maximum after 48 hours. Barron (3) found reduced respiration with 200 r; van Bekkum (4) reported a decrease after irradiation, with as small a dose as 100 r. Conversely, mouse spleen seemed unaffected by 640 r (5). Szuszko (6) found an increase in O₂ tension in rabbit brain with doses of 900 r, whereas Flosheim, Doernback, and Morton (7) reported no significant changes in mouse brain respiration with doses as high as 16,000 r. Barron (3) saw little effect on rat liver respiration with 800 r, whereas Kusnets (1) found considerable reduction in O₂ uptake. Hanel, Hjort, and Purser (8) reported an increased respiration in liver from rats exposed to 450 r of whole body irradiation. Muscle and kidney tissue showed almost no respiratory effects with doses as high as 800 r (1).

Potter and Bethell (9) showed a reduction in oxidative phosphorylation in spleen mitochondria subjected to 800 r in vivo. Dubois and Paterson (10) thought that this reduction was due to increased ATPase activity, but this was disputed by Ashwell and Hickman (5) who used fluoride as an ATPase inhibitor. Goldfeder (11) demonstrated a decrease in oxidative phosphorylation in mouse liver mitochondria with 700 r of whole body irradiation, but van Bekkum (12) found no reduction in various animal tissues. Kusnets (1) reported a decrease in O₂ tension in rat liver mitochondria from rats exposed to considerably over 700 r, and Toropova (13) reported a decrease in ATP synthesis in liver homogenates from rats exposed to 650 r.

Because of the conflicting evidence and the variety of preparations and dosages reported to date it was thought advisable to reinvestigate the problem using carefully reproducible doses, various substrates, and mitochondria prepared under rigidly controlled conditions. Oxidative phosphorylation was measured at intervals up to 72 hours after whole body irradiation.

**Experimental Procedure**

**Irradiation**—Sherman strain male rats weighing 200 to 250 g were placed in a Lucite restraining cage and subjected to 840 r of whole body x-irradiation, delivered at a dose rate of 120 r per minute in air. The x-ray unit was set at 90 kv, 5 ma, and had a 2-mm aluminum filter. The radiation entered the rats ventrally and the target distance was 7 inches. Exact measurements with a dosimeter indicated that 550 r reached the liver. The control group was subjected to the same conditions except for the actual irradiation. Before and after the irradiation the animals were maintained on Purina laboratory chow and fed water ad libitum.

**Preparation of Mitochondria**—At various time intervals after irradiation, the rats were killed by decapitation with a guillotine and exsanguinated. The liver was removed and placed immediately in ice-cold isotonic sucrose-Versene (ethylenediaminetetra-acetate) (0.25 m sucrose; 0.005 m Versene). The mitochondria were then extracted by a method described previously (14). Spleen weights for control and irradiated animals were recorded.

**Analysis**—Oxidative phosphorylation was measured in 15-ml Warburg vessels maintained in a water bath at 30° for a period of 90 minutes. The vessels were kept in an ice bath during preparation and the mitochondrial suspension was added last. Ten minutes of equilibration were allowed in the Warburg bath. The reaction was then initiated by tipping in the hexokinase-glucose trap from the side arm. After 20 minutes the reaction was stopped by the rapid addition of ice-cold 5% trichloroacetic acid.

The standard reaction medium had a final volume of 2.2 ml and contained: Tris buffer, at pH 7.4, 0.07 M; inorganic phosphate, 30 or 40 μmoles; MgCl₂, 0.005 M; cytochrome c, 10⁻⁶ M; ATP (Na salt), 0.002 M; Na-pyruvate, 0.025 M; (or β-hydroxybutyric acid (Na salt), 0.025 M; Na-citrate, 0.025 M; DL-glutamic acid (Na salt), 0.025 M); 0.4 or 0.5 ml of mitochondrial suspension containing 5 to 8 mg of protein; and a phosphate trap consisting of 5 mg of hexokinase (750 units) and 24 μmoles of glucose. The protein-free filtrate was analyzed for inorganic phosphate by the method of Lowry and Lopez (15). Mitochondrial protein was determined by the biuret method (10). Counting of a sample of fresh mitochondrial suspension was done in a Petroff-Hauser counting chamber under phase-contrast microscopy at 1200× magnification by the method of Shelton, Schneider, Streibich (17). The samples of cytochrome c, ATP, and hexokinase (practical type III) were obtained from the Sigma Chemical Company.

**Results**

The protein content of the mitochondrial suspension and the number of mitochondria present are shown in Table I. The

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1 X-ray unit and dosimeter were calibrated by C. B. Braestrup, 650 West 188th Street, New York 32, New York.
TABLE 1
Estimation of liver mitochondria

<table>
<thead>
<tr>
<th>Animal groups: hours post radiation</th>
<th>Protein per ml of suspension (mg)</th>
<th>Count per ml of suspension (× 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (13)</td>
<td>15.8 ± 0.4</td>
<td>78.5 ± 4.1</td>
</tr>
<tr>
<td>1-Hour (7)</td>
<td>14.0 ± 0.7</td>
<td>63.5 ± 4.1</td>
</tr>
<tr>
<td>3-Hour (8)</td>
<td>12.4 ± 0.9</td>
<td>60.0 ± 1.5</td>
</tr>
<tr>
<td>6-Hour (7)</td>
<td>11.7 ± 0.8</td>
<td>72.5 ± 7.1</td>
</tr>
<tr>
<td>12-Hour (6)</td>
<td>13.3 ± 0.6</td>
<td>77.5 ± 5.2</td>
</tr>
<tr>
<td>24-Hour (4)</td>
<td>15.6 ± 1.3</td>
<td>65.0 ± 6.8</td>
</tr>
<tr>
<td>48-Hour (5)</td>
<td>13.0 ± 1.5</td>
<td>75.0 ± 3.0</td>
</tr>
<tr>
<td>72-Hour (5)</td>
<td>14.2 ± 1.2</td>
<td>72.5 ± 3.5</td>
</tr>
</tbody>
</table>

The mitochondrial count did not follow such a consistent pattern. There was a slight fall and a more rapid recovery, but the significance of these variations is questionable. Mitochondria from irradiated animals were more fragile, disintegrating in the counting chamber within 15 minutes, whereas normal mitochondria appeared relatively unchanged after 1 hour or more.

The capacity for oxidative phosphorylation of the mitochondria isolated from animals at various time intervals after irradiation is shown in Table II. The data show the same general trends for all four substrates. In the first 3 hours following irradiation there was a rapid decrease of about 25% in the P:O ratios. This impairment in oxidative phosphorylation lasted for the next 9 hours and then began to improve. The P:O levels of the control were reached with mitochondria isolated within 24 hours from irradiated animals and tested in the presence of pyruvate, β-hydroxybutyrate, or citrate; with glutamate as substrate recovery required 72 hours. P:O ratios were lowest with pyruvate and β-hydroxybutyrate after 3 hours; with citrate and glutamate after 6 hours. The reasons for the decrease in P:O ratios are obvious: phosphate uptake decreased within 1 hour by about 20% and remained at that level through out the first 12 hours. Oxygen consumption, however, did not fall significantly. On the contrary, values found in the 6-hour group were significantly higher than these for the control rate, except when pyruvate was the substrate. Recovery occurred between 12 and 24 hours after irradiation. In the group killed after 24 hours, oxygen consumption had returned to control levels with all substrates. Phosphate uptake reached approximately the control levels at this time too, except when glutamate was used as substrate, and in this instance 48 hours were needed for full recovery. Both oxidation and phosphorylation were higher in the “irradiated” mitochondria than in the control after 48 hours for all substrates except pyruvate, but this was no longer true after 72 hours.

The spleens of the irradiated animals showed the expected decrease in weight (18). The average weights of the spleens from the different groups were: control, 565 g; 1 and 3 hours, 460 g; 6, 12, and 24 hours, 360 g; 48 hours, 290 g; and 72 hours, 225 g. Thus the spleens in the last group had lost 60% of their weight.

DISCUSSION

Many of the conflicting reports on the effects of whole body irradiation on oxidative phosphorylation by liver, and other tissues, appear to be due to two major factors: (a) the amount of ionizing radiation actually reaching the organ and (b) the length of time after irradiation at which the effect is measured. Roentgen measurements taken with a dosimeter embedded at the level of the liver disclosed that the amount of ionizing radiation reaching that area varied greatly with the size of the animal and with the position of the animal during exposure in air. The degree of radiation damage is known to vary widely from one tissue to another. Even in a relatively resistant organ, such as the liver, it is obvious from the data presented here that an adequate evaluation of x-ray damage cannot be obtained with one substrate, one arbitrarily chosen time interval after exposure, or one criterion such as oxygen consumption.

It has been shown clearly that the efficiency of phosphorylation is impaired in mitochondria from irradiated animals. The greatest decrease in efficiency was seen 3 to 12 hours after exposure, the P:O ratios returning to normal within 24 hours in most cases. This biochemical lesion paralleled the decrease and subsequent increase in the protein content of the mitochondria. It is logical to conclude that a chemical or structural change occurred in the mitochondrial membrane. It is well known that the coupling of phosphorylation to oxidation is dependent upon the integrity of the mitochondrial membrane, and that it is impaired by any chemical or physical agent which affects the membrane. The fact that tremendous doses of irradiation do not cause comparable damage to isolated mitochondria...
TABLE II

Oxidative phosphorylation in rat liver mitochondria

<table>
<thead>
<tr>
<th>Animal groups: represent hours post radiation</th>
<th>Pyruvate</th>
<th>β-Hydroxy butyrate</th>
<th>Citrate</th>
<th>Glutamate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O₂ uptake</td>
<td>ATP formed</td>
<td>P:O</td>
<td>O₂ uptake</td>
</tr>
<tr>
<td>Control (48)</td>
<td>24.6 ± 0.9</td>
<td>32.3 ± 1.7</td>
<td>2.1 ± 0.06</td>
<td>24.6 ± 0.5</td>
</tr>
<tr>
<td>1-Hour (24)</td>
<td>22.5 ± 0.9</td>
<td>40.0 ± 1.3</td>
<td>1.8 ± 0.15</td>
<td>24.3 ± 0.5</td>
</tr>
<tr>
<td>NS</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.1</td>
<td>35.0 ± 0.8</td>
<td>81.5 ± 2.3</td>
</tr>
<tr>
<td>3-Hour (28)</td>
<td>21.6 ± 1.2</td>
<td>31.3 ± 2.1</td>
<td>1.6 ± 0.00</td>
<td>28.8 ± 0.8</td>
</tr>
<tr>
<td>6-Hour (20)</td>
<td>27.1 ± 0.6</td>
<td>42.5 ± 3.4</td>
<td>1.6 ± 0.08</td>
<td>32.3 ± 0.7</td>
</tr>
<tr>
<td>12-Hour (16)</td>
<td>25.1 ± 1.3</td>
<td>41.3 ± 2.2</td>
<td>1.7 ± 0.05</td>
<td>30.2 ± 1.0</td>
</tr>
<tr>
<td>24-Hour (15)</td>
<td>22.3 ± 1.4</td>
<td>49.0 ± 1.5</td>
<td>2.2 ± 0.06</td>
<td>22.2 ± 1.3</td>
</tr>
<tr>
<td>48-Hour (16)</td>
<td>22.0 ± 0.0</td>
<td>48.2 ± 2.9</td>
<td>2.2 ± 0.14</td>
<td>28.3 ± 0.8</td>
</tr>
<tr>
<td>72-Hour (16)</td>
<td>24.6 ± 1.7</td>
<td>50.0 ± 2.2</td>
<td>2.2 ± 0.13</td>
<td>27.4 ± 1.0</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

(12) points to an effect at the cellular level. Krebs (19) has suggested that x-rays impair the functioning of "pacemaker" reactions and, thus, cellular metabolism as a whole. This would undoubtedly affect the chemical composition of mitochondria and hence their biochemical function.

**SUMMARY**

1. Mitochondria were isolated from the livers of rats exposed to a single acute dose (840 r) of whole body irradiation at 1, 3, 6, 12, 24, 48, and 72 hours before they were killed, and from sham-treated controls.

2. Mitochondrial protein fell to 75% of the control level within 6 hours after irradiation and returned to normal within 24 hours.

3. The number of mitochondria decreased to some extent during the first 3 hours and then recovered, but the significance of these variations is questionable. Irradiated mitochondria show greater size variation and fragility.

4. O₂ consumption varied considerably during the time period investigated. It was higher than the control rate with all four substrates 6 hours after irradiation, and returned to normal after 24 hours.

5. The rate of synthesis of adenosine triphosphate was decreased by 20% after irradiation for 1 hour and remained at this lower level for 12 hours. It had returned to the control level by 24 hours in most cases and by 48 hours in all.

6. P:O ratios were reduced by 25% 3 hours after irradiation and remained so for 12 hours. Control levels were reached with three substrates within 24 hours, but with glutamate only after 48 hours.

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